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(71) Applicant *(for all designated States except US)*: **AMNIS CORPORATION** [US/US]; 2505 Third Avenue, Suite 210, Seattle, WA 98121 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GEORGE, Thaddeus, C.** [US/US]; 1712 NW 63rd Street, Seattle, WA 98107 (US). **BASIJI, David, A.** [US/US]; 6538 Greenwood Avenue N., Seattle, WA 98103 (US). **FROST, Keith** [US/US]; 4801B South Roxbury Street, Seattle, WA 98118 (US). **HALL, Brian, E.** [US/US]; 1121 17th Avenue, Apt. A, Seattle, WA 98122 (US). **ORTYN, William, E.** [US/US]; 11546 Matsu Place NE, Bainbridge Island, WA 98110 (US). **SEO, Michael, J.** [US/US]; 8120 SE 77th Place, Mercer Island, WA 98040 (US).

(74) Agents: **HERMANNS, Karl, R.** et al.; Seed Intellectual Property Law Group PLLC, Suite 6300, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).

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(54) Title: IMAGE BASED QUANTITATION OF MOLECULAR TRANSLOCATION

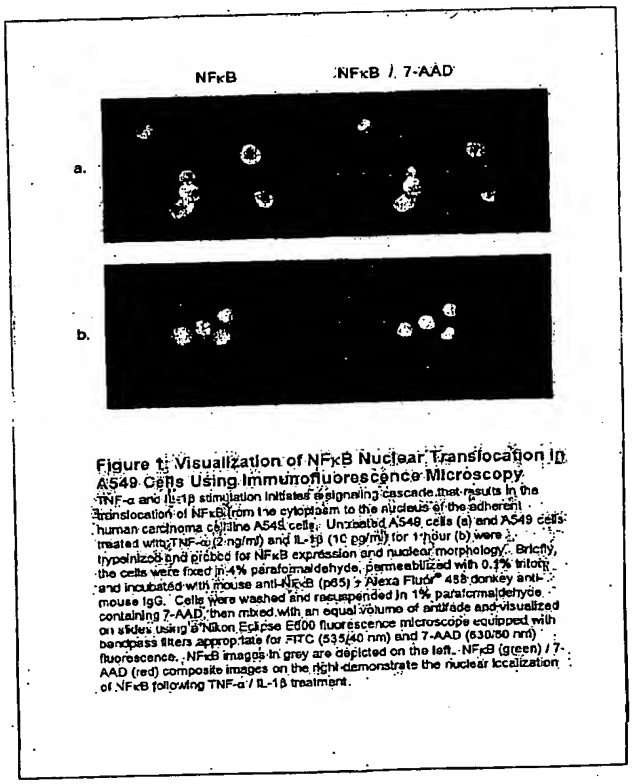

Figure 1: Visualization of NFκB Nuclear Translocation in A549 Cells Using Immunofluorescence Microscopy. TNF-α and IL-1β stimulation initiates a signaling cascade that results in the translocation of NFκB from the cytoplasm to the nucleus of the adherent human carcinoma cell line A549 cells. Untreated A549 cells (a) and A549 cells treated with TNF-α (2 ng/ml) and IL-1β (10 pg/ml) for 1 hour (b) were trypsinized and probed for NFκB expression and nuclear morphology. Briefly, the cells were fixed in 4% paraformaldehyde, permeabilized with 0.1% triton and incubated with mouse anti-NFκB (p65) / Alexa Fluor® 488 donkey anti-mouse IgG. Cells were washed and resuspended in 1% paraformaldehyde containing 7-AAD, then mixed with an equal volume of antifade and visualized on slides using a Nikon Eclipse E600 fluorescence microscope equipped with bandpass filters appropriate for FITC (535/40 nm) and 7-AAD (630/50 nm) fluorescence. NFκB images in grey are depicted on the left. NFκB (green) / 7-AAD (red) composite images on the right demonstrate the nuclear localization of NFκB following TNF-α / IL-1β treatment.

(57) Abstract: The use of a multi-spectral imaging system, cell compartment markers, and molecular probes in a method for measuring movement of molecules within a cell by correlation analysis is provided, including measuring molecular movement to a particular compartment in adherent and non-adherent cells, e.g. in response to biological stimuli. A compartment in the cell is defined by the image of a specific compartment marker, e.g., a nuclear fluorescent stain. Molecule location is provided by a probe labeled with a different fluorochrome. A mask is generated based on the compartmental marker, and a correlation measurement is made between the locations of the molecular probe and the compartment marker. The correlation value between the regions defined by the compartment mask and molecular probe gives a quantitative measurement of the translocation of the molecule. The use of only a single masking function simplifies measurement of molecular translocation within a cell.

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